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# The adsorption of bovine serum albumin by liposomes

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## Summary

The adsorption of bovine serum albumin on positively charged, negatively charged and neutral liposomes with different ratios of charge has been investigated. It was found that the amount adsorbed at saturation was not influenced by the pH, characteristics of charge or ratio of charge. This may be due to the driving force for adsorption being dominated by hydrophobic effect.

## Introduction

Several reports dealing with the interaction of albumin with liposomes have been published (Sweet and Zull, 1970; Kimelberg and Papahadjopoulos, 1971; Papahadjopoulos et al., 1975; Kimelberg, 1976; Kitagawa et al., 1976; Lis et al., 1976; Hoekstra and Scherphof, 1979).

For drug delivery systems, positively charged, negatively charged and neutral liposomes have been used (Knight, 1981). It is therefore of particular interest to know the influence of charge characteristics of liposomes on the interaction with serum albumin. The work reported here is an investigation of the adsorption of bovine serum albumin on positively charged, negatively charged and neutral liposomes with different ratios of charge. The effect of pH on adsorption is also demonstrated.

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## Materials and Methods

Crystallized and lyophilized bovine serum albumin (essentially fatty acid and globulin free) was purchased from Sigma Co. (U.S.A.) and used without further purification. Cholesterol was obtained from Sigma Co. (U.S.A.). Dicetyl phosphate and stearylamine were purchased from P.L. Biochemical (U.S.A.). Phosphatidylcholine was prepared according to the methods of Hanahan et al. (1951) and Singleton et al. (1965) with modification by using a two-column procedure of alumina column and silicic acid column. Purity of the isolated phosphatidylcholine was checked by thin-layer chromatography (TLC alumina sheets silica gel 60, Merck, F.R.G.). The solvent system was chloroform–methanol–water (65 : 25 : 4 v/v).

One characteristic spot was developed by Dragendroff reagent. No spots were detected when ninhydrin reagent and iodine vapors were used.

*Preparation of liposomes*

A chloroform mixture of phosphatidylcholine, cholesterol and either dicetyl phosphate or stearylamine at the desired molar ratio was dried to a thin film under reduced pressure at 37°C in a rotary evaporator. Multilamellar liposomes were prepared by constant vortexing for 5 min in 0.9% sodium chloride solution to give a lipid concentration of 13.6 mmol. The liposome preparation was hydrated at 37°C for 2 h.

*Adsorption with bovine serum albumin*

Equal volumes of liposome dispersion and bovine serum albumin solution were mixed and equilibrated in a constant shaking water bath (100 throws per minute) at a temperature of 37°C. The time necessary to establish equilibrium was found to be 90 min. The pH of the dispersions was adjusted to the required value by means of hydrochloric acid and/or sodium hydroxide solution. In this study, a high concentration of liposomes was used to increase sensitivity of adsorption.

*Analytical method*

The mixture after equilibrium was centrifuged at 140,000 × *g* for 2 h to give a clear supernatant. Determination of bovine serum albumin concentrations was made by the Lowry method (Lowry et al., 1951; Hartree, 1972). High concentrations of bovine serum albumin were diluted as necessary. Calibration curves were linear over the concentration range studied.

## Results and Discussion

Liposome incorporation with different molar ratios of dicetyl phosphate was investigated by microelectrophoresis. It was found that at a molar ratio of 0.15 the liposomes showed a mobility of $-1.3\ \mu m \cdot s^{-1}$ per $V \cdot cm^{-1}$ which gave a zeta potential of −16.7 mV. This approximates the mobility of human red blood cells. It is proposed that a negative liposome with a charge near that of red blood cells is suitable for a drug delivery system. Liposome incorporation with dicetyl phosphate at a molar ratio of 0.2 showed a mobility of $-1.7\ \mu m \cdot s^{-1}$ per $V \cdot cm^{-1}$ which is equivalent to a zeta potential of −21.2 mV. Liposomes with this charge content are sufficiently high for the study of pH effect on the adsorption of bovine serum albumin. The effect of pH on the adsorption of bovine serum albumin on liposomes is shown in Fig. 1. It is clear that in the pH range studied, the amount of bovine serum albumin adsorbed on the positively charged, negatively charged and neutral liposomes is not influenced by the pH. It can be suggested that the charge effect of bovine serum albumin molecules and the charge characteristics of liposomes are not factors affecting adsorption.

The adsorption of serum albumin on negatively charged latex models has shown that near the isoelectric point, the amount adsorbed increased to a maximum (Morrissey and Stromberg, 1974; Norde and Lykelma, 1978; Suzawa and Murakami, 1980). This is probably because at the isoelectric point, repulsion between adsorbed molecules is at a minimum. Protein molecules adopt a flatter conformation to facilitate adsorption. However, this is not the case with liposomes. The adsorption conformation of bovine serum albumin on liposomes may differ from that on lattices. In addition, the driving forces for bovine serum albumin adsorbed on liposomes and lattices may be

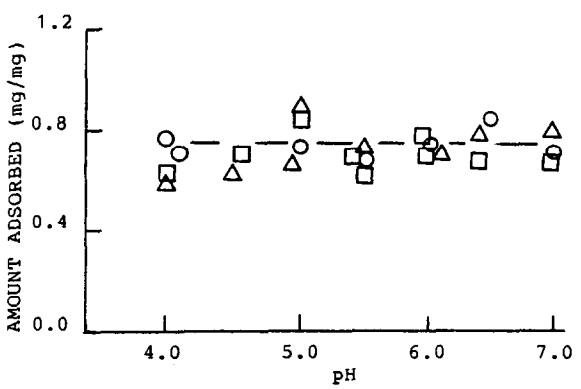


Fig. 1. Effect of pH on adsorption of bovine serum albumin by liposomes. Phosphatidylcholine/cholesterol/stearylamine (1.6 : 1 : 0.2), ○; phosphatidylcholine/cholesterol/dicetyl phosphate (1.6 : 1 : 0.2), □; phosphatidylcholine/cholesterol (1.6 : 1), △.

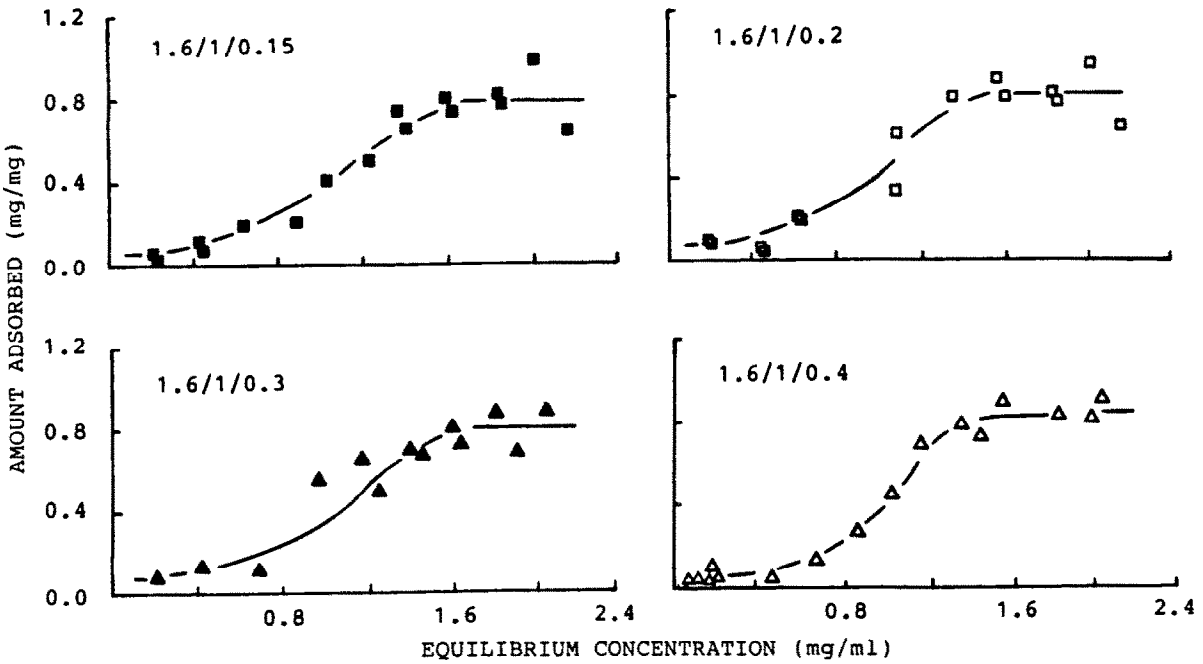


Fig. 2. Adsorption of bovine serum albumin by positively charged liposomes (phosphatidylcholine/cholesterol/stearylamine).

different. For example, bovine serum albumin adsorption on lattices may be due to both electrostatic interaction and hydrophobic interaction (Norde and Lykelma, 1978; Suzawa and Murakami, 1980). Whereas for bovine serum albumin adsorbed on liposomes hydrophobic interaction may dominate, Kimelberg (1976) pointed out that the hydrophobicity of serum albumin was not altered by changing the pH. If bovine serum albumin is adsorbed on liposomes mainly via hydrophobic interaction, it is not surprising that the amount adsorbed does not change at different pH.

The adsorption of bovine serum albumin onto positively charged, negatively charged and neutral liposomes is given in Figs. 2, 3 and 4, respectively, by plotting the amount of bovine serum albumin adsorbed against the equilibrium concentration of bovine serum albumin after adsorption. All curves show a similar adsorption pattern of bovine serum albumin on positively charged, negatively charged and neutral liposomes in the concentration range of bovine serum albumin studied, i.e. there is an increase in bovine serum albumin adsorption as concentration increases until a saturated region is attained. It is interesting to find that the adsorption of bovine serum albumin on positively charged, negatively charged and neutral liposomes gives the same value at saturation, despite the ratio of charge and characteristics of charge of liposomes, in the range of ratio of charge studied.

The binding of bovine serum albumin onto liposomes was attained at pH 5.0 which is near the isoelectric point of bovine serum albumin where molecules acquire equal positive and negative charges. If bovine serum albumin interacted with positively charged or negatively charged liposomes through electrostatic charge interaction, the saturated amount adsorbed of bovine serum albumin would increase as the ratio of charge of liposomes increased. However, this is not the case. Looking at the saturated amount adsorbed of bovine serum albumin on neutral liposomes (Fig. 4), we see the same value as those of positively and negatively charged liposomes. Obviously, the bovine serum albumin interaction with liposomes did not go through an electrostatic charge interaction. As suggested by Papahadjopoulos et al. (1975), hydrophobic interaction may play an im-

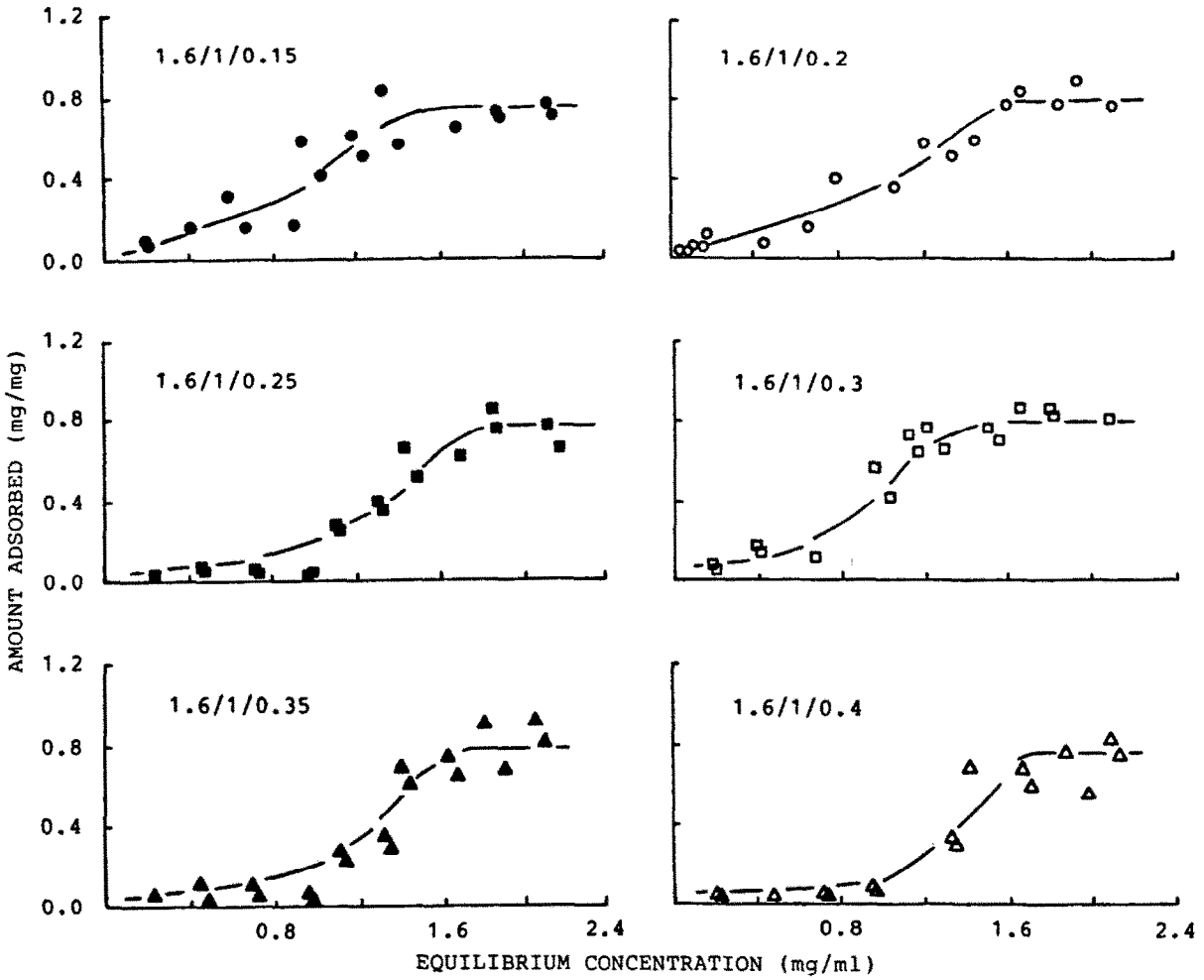


Fig. 3. Adsorption of bovine serum albumin by negatively charged liposomes (phosphatidylcholine/cholesterol/dicetyl phosphate).

portant role in bovine serum albumin adsorption on liposomes.

At initial adsorption, bovine serum albumin molecules adsorbed onto the liposome surface may be due to the charge effect for charged surface and van der Waals forces, ion-dipole forces and hydrogen bonds for neutral surface. In low bovine serum albumin concentrations, the mutual influence of the adsorbed molecules is little and they may adopt a flatter conformation on the surface or attach on the surface by points. Only a small amount of bovine serum albumin molecules may penetrate into the phospholipid bilayer. As con-

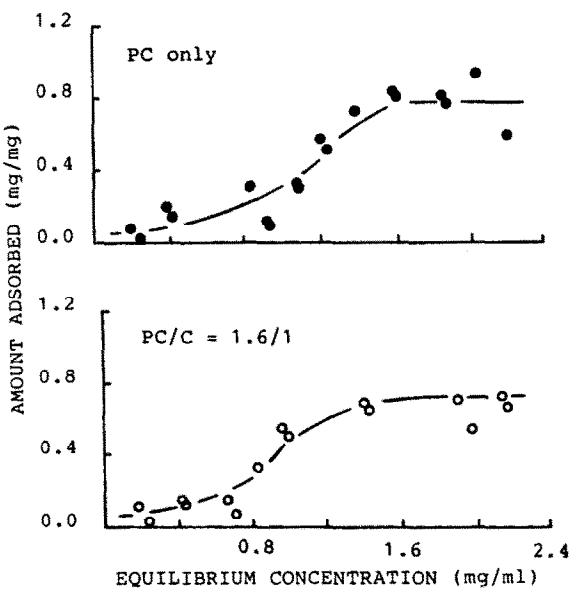


Fig. 4. Adsorption of bovine serum albumin by neutral liposomes. Phosphatidylcholine, ●; phosphatidylcholine/cholesterol (1.6:1), ○.

centration increases, more molecules are adsorbed, their conformations are rearranged into a denser molecular packing or aggregation occurs on the surface. Most of the albumin molecules penetrate the phospholipid bilayer and bind to the hydrophobic region of the phospholipid molecules by hydrophobic interaction which is the dominant force for adsorption.

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